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Prenatal Diagnosis of Fukuyama Type Congenital Muscular Dystrophy by Polymorphism Analysis

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Fukuyama type congenital muscular dystrophy (FCMD) is an autosomal recessive disorder characterized by a combination of primary muscular dystrophy of early infantile onset and brain malformation (lissencephaly type II). The identification of the FCMD gene locus at 9q31 opened the theoretical possibility of prenatal diagnosis. The authors conducted prenatal diagnosis in two unrelated FCMD families by analysis using nine microsatellite CA-repeat polymorphic markers flanking the FCMD locus, and calculated phenotype probabilities in fetuses with a computer program, LINKAGE. The fetus in family 1 showed a 99% probability of being healthy either as a normal homozygote or a heterozygote carrier and was born without signs of FCMD. In family 2, the fetus was diagnosed to have FCMD with at least 86% probability. The parents of this family decided to terminate the pregnancy and an abortus showed brain malformations characteristic of an FCMD fetus.
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KEY WORDS: prenatal diagnosis, Fukuyama type congenital muscular dystrophy, polymorphism analysis, brain anomaly

INTRODUCTION

Fukuyama type congenital muscular dystrophy (FCMD), first described by Fukuyama et al. [1960] and entered in the MIM (Mendelian Inheritance in Man) catalog as No. 253800 [McKusick, 1994], is characterized by brain malformations, principally cerebral and cerebellar cortical dysplasia, and dystrophic changes in the skeletal muscle [Nonaka and Chou, 1979; Fukuyama et al., 1981]. Clinical manifestations of the disease are unique; generalized hypotonia and weakness are present in infancy, followed by marked muscle atrophy, joint contractures, and psychomotor developmental delay in childhood. The highest motor function acquired by most patients is sliding while sitting on their buttocks. Upright ambulation, even with support, is attained only rarely in exceptional cases. Intellectual, cognitive, and communicative functions are moderately delayed without exception, while febrile and/or nonfebrile seizures occur in about half of cases. The overall clinical course is inexorably progressive with average age at death of 16 years. No treatment is available. Judging from the high prevalence of consanguineous parental marriages and sib recurrence, and from the absence of sexual preference and vertical transmission, simple autosomal recessive mode of inheritance was proposed [Fukuyama et al., 1960]. This hypothesis was supported by detailed pedigree analyses [Osawa, 1978; Fukuyama and Osawa, 1984], demonstrating a segregation ratio in 153 FCMD sibships not significantly different from 0.25. Toda et al. [1993] recently succeeded in mapping the FCMD locus to 9q31-q33 by genetic linkage analysis. In addition, the same research group further narrowed the locus to a region very close to a marker, mfd220 [Toda et al., 1994]. This discovery opened the door to the long-awaited possibility of prenatal diagnosis for FCMD families.

Here, we report successful prenatal diagnosis of two families by polymorphism analyses with microsatellite markers flanking the FCMD locus.

CLINICAL REPORTS

Family 1

Parents were not consanguineous nor were any relatives affected other than the propositus. The patient (II-2, see Fig. 2) of family 1, the second child, was floppy at birth. At age 10 months, a diagnosis of FCMD was made at another hospital because of a high serum creatine kinase (CK) value (13,970 mU/ml), muscle biopsy findings of necrotic, regenerating muscle fibers, and markedly increased endomysial and perimysial connective tissues. When the patient was 4½ years old, the family visited our hospital with the aim of possibly ob-

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taining a prenatal diagnosis. The patient was profoundly retarded in psychomotor development, could slide while sitting, but could not stand or speak any meaningful words. Facial muscle involvement, multiple joint contractures, and absent deep tendon reflexes were noted. Brain MRI at age 4²⁄₁₂ years documented pachygyric frontal lobes, polymicrogyric occipital lobes, and a myelination delay in the same regions (Fig. 1a).

Family 2

The proposita (II-3, Fig. 2) of family 2 was born to non-consanguineous parents, and recognized to be hypotonic and weak at age 5 months. At age one year, a diagnosis of FCMD was made at another hospital based on clinical findings including a high CK value (4,015 m U/ml) and dystrophic muscle changes in a biopsy specimen. A febrile convulsion occurred at age 20 months. When first seen by us at age 2 years, the patient was able to maintain a sitting posture and to play with toys but could not slide while sitting or standing. Generalized hypotonia and weakness, a myopathic face, and mild knee contractures were noted. The patient had no vocabulary. Brain MRI at age 2¹⁄₁₂ years demonstrated pachygyria in frontal and occipital lobes widely dilated Sylvian fissures (operculum dysplasia), mild dilatation of the posterior horns of the lateral ventricles, and delayed myelination in centro-temporal subcortical regions (Fig. 1b).

Genetic Counseling for Prenatal Diagnosis

We were consulted by both couples regarding prenatal diagnosis after a third child was conceived in family 1, and a fourth in family 2. Family 1 came to us specifically, because the parents were under a great deal of pressure from the paternal grandparents to terminate the pregnancy. The parents thought prenatal diagnosis as the only hope of avoiding this unwanted termination. After the nature of the procedure and the safety

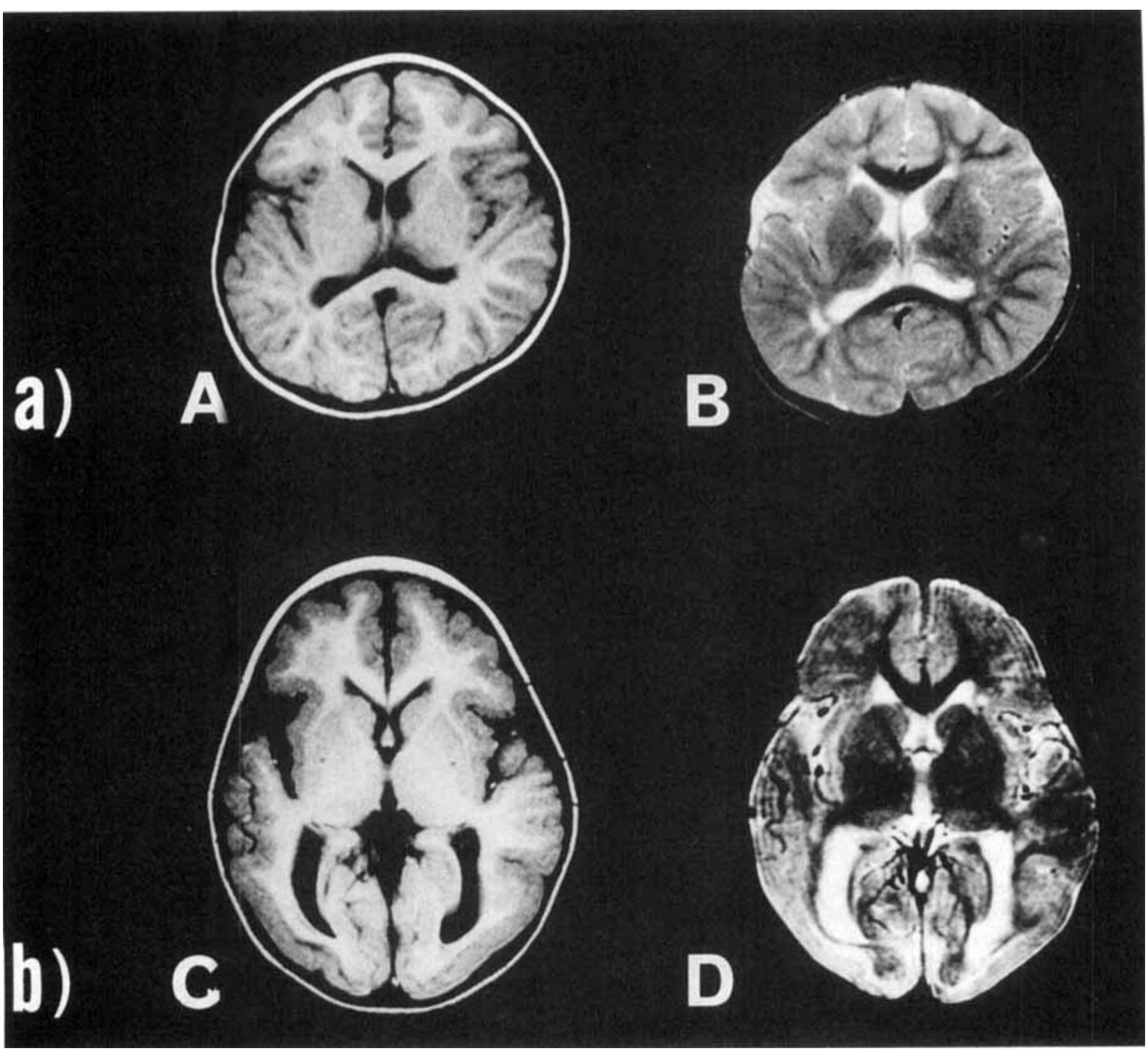


Fig. 1. Brain MRI of the probands of family 1 (**a**) and of family 2 (**b**). A and C are T1-weighted inversion-recovery images, and B and D T2-weighted spin-echo images. Delayed myelination, mild ventricular enlargement, dilated Sylvian fissures (operculum dysgenesis) and pachygyria are evident.

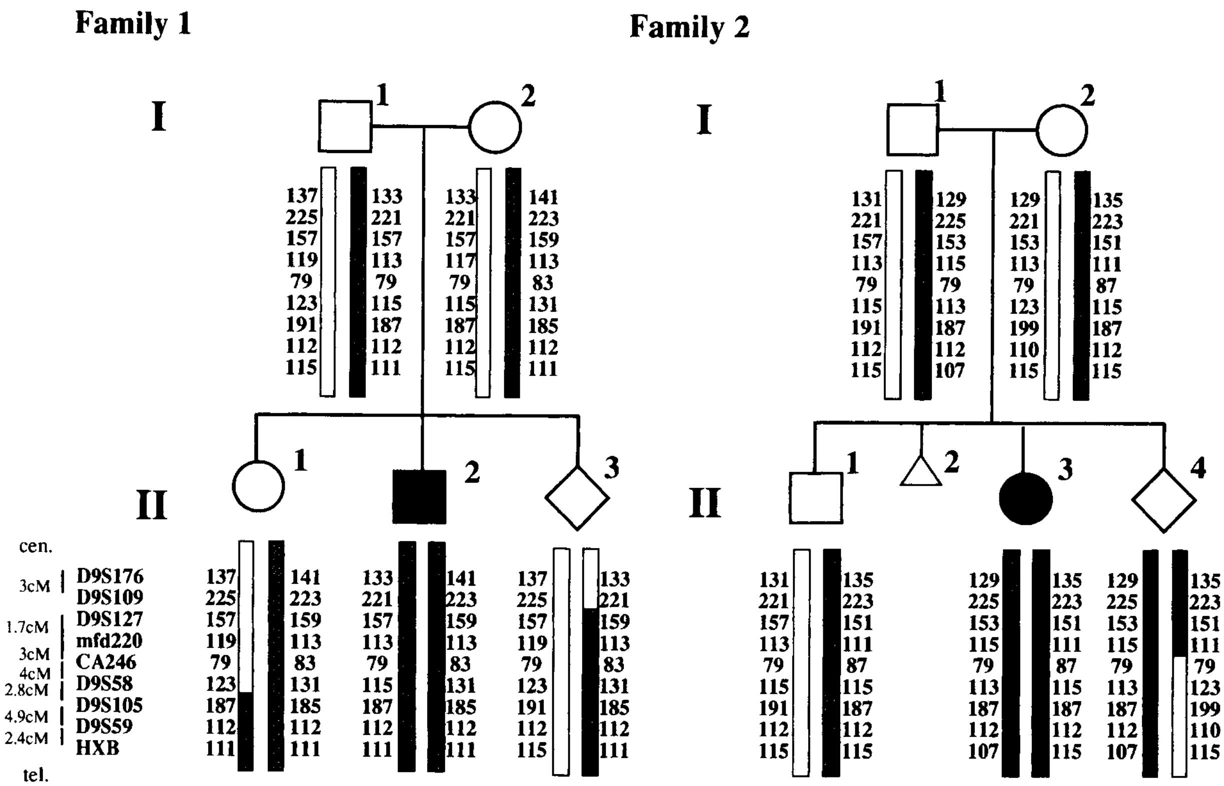


Fig. 2. Nine loci genotypes from members of the two pedigrees. The FCMD gene is presumed to lie between D9S127 and CA246 loci. The mfd220 is the closest marker. Values shown as haplotypes are PCR-product sizes. Shaded and unshaded columns depict the regions carrying the mutant and wild-type FCMD alleles, respectively. In the fetus (II-3) of family 1, the paternally derived chromosome carries the wild-type allele, while the maternally derived chromosome carries the mutant allele, irrespective of occurrence of a cross-over at a site proximal to D9S127. The fetus (II-4) in family 2 inherited a paternal allele carrying the mutation. In the maternal allele, a crossover was identified between the mfd220 and CA246 loci.

and risks of examination had been explained and the confidentiality of all results was guaranteed, parents of both families expressed their eagerness to choose prenatal diagnosis. We obtained approval from the Intramural Ethical Committee and this protocol was conducted according to the Prenatal Diagnosis Guidelines from the Japan Society of Human Genetics. At the time of amniocentesis for the fetal diagnosis, the patients in families 1 and 2 were 4½ and 2²⁄₁₂ years old, respectively.

MATERIALS AND METHODS

Procedures in Prenatal Diagnosis

DNA was extracted from peripheral blood leukocytes of patients, their sibs and parents according to the standard techniques [Sambrook et al., 1989]. Amniocentesis was performed under ultrasonography at 17 gestational weeks in family 1 and at 16 weeks in family 2. The amniotic fluid was then portioned into two halves. DNA was extracted directly from one half and from cultured amniotic cells grown from the other half. Haplotypes of all samples from relatives and both fetuses were analyzed using polymorphic microsatellite markers located at 9q31-q33, as described below.

Polymorphism Analysis

We used nine polymorphic microsatellite (CA repeat) markers, previously mapped to 9q31-q33 [NIH/CEPH collaborative mapping group, 1992; Weissenbach et al., 1992]. The markers/loci used included D9S176 [Weissenbach et al., 1992], D9S109 [Furlong et al., 1992], D9S127 [Lyall et al., 1992], mfd220 [Weber in GDB], CA246 [Toda et al., 1995], D9S58 [Kwiatkowski et al., 1992], D9S105 [Wilkie et al., 1992], D9S59 [Kwiatkowski et al., 1992], and HXB [Ozelius et al., 1992] (Fig. 2). The FCMD locus is considered to lie within a few hundred kilobases of the mfd220 locus [Toda et al., 1994]. Primer sets for polymerase chain reaction (PCR) amplification of these marker DNAs were synthesized.

Conditions for PCR and subsequent electrophoresis conditions were as previously described [Toda et al., 1993, 1994]. Genomic DNA (20 ng) from each sample was amplified by PCR using 20 pmol of one unlabeled primer and 20 pmole of one primer end-labeled with 1.0 μCi [γ-^{32}P] ATP using T4 polynucleotide kinase/1 × PCR buffer (16.6mM NH_4SO_4, 67 mM Tris-HCl, pH 8.8, 10 mM β-mercaptoethanol, 6.7 μM EDTA)/10% (v/v) dimethyl sulfoxide/1.5 mM of each dNTP/5 mM $MgCl_2$/1.25 U Taq DNA polymerase. Samples were

incubated in a DNA thermocycler (Perkin Elmer Cetus, Norwalk, CT) for 35 cycles under the following conditions: 94°C for 1.5 min, 55°C for 2 min, and 72°C for 1.5 min. PCR products were analyzed on 6% polyacrylamide gel and visualized by autoradiography.

Haplotyping and Calculation of Phenotype Possibility in Fetuses

Based on the genotype data, a most likely linkage phase was assumed, haplotypes were constructed, and the fetal phenotypes were estimated. Phenotype probabilities in fetuses were calculated with a computer program, LINKAGE (MLINK) [Lathrop et al., 1984]. For the calculation, we chose the genotype data for D9S127, mfd220, and D9S58 loci, all of which are known to be close to the FCMD locus and have been identified as reliable loci [Toda et al., 1993; 1994]. Risk calculation using the CA246 locus was not feasible because it was tentatively mapped on the basis of both recombination events and recombination fraction (Qmax) showing the maximum lod score in the FCMD linkage analysis [Toda et al., 1994, 1995]. Calculation was based on two distinct possibilities for the FCMD locus: the order and distance of the marker and FCMD loci was arranged either as (1) cen - D9S127 - (1cM) - FCMD - (1cM) - mfd220 - (7cM) - D9S58 - tel, or as (2) cen - D9S127 - (2cM) - mfd220 - (1cM) - FCMD - (6cM) - D9S58 - tel (Fig. 3).

RESULTS

In family 1, the fetus had a paternally derived chromosome which may not have carried the FCMD gene mutation, while the other chromosome derived from the mother had a crossover. The maternally derived haplotype for markers distal to D9S127 including the mfd220 locus was identical to that of the patient (Fig. 2). In family 2, the fetus inherited a paternal chromosome carrying the mutation, whereas in the maternal chromosome, a crossover was identified between the mfd220 and CA246 loci. The haplotype proximal to the mfd220 locus was identical to that of the patient. Phenotype probability calculated on the basis of both assumption modes indicated that the fetus in family 1 was an FCMD carrier with at least 99% certainty. The calculation in family 2 showed that the fetus was affected with 99% probability when localization of the FCMD gene was assumed to be proximal to the mfd220 locus, and with 86% probability if the gene was located distally to this locus (Fig. 3).

The parents of both families were informed of the above results at 19 gestational weeks. The parents in family 1 decided to continue the pregnancy and a healthy girl was born. She is currently 10 months old and has shown no signs suggestive of FCMD. Her serum CK level was 120 mU/ml. In family 2, the parents opted for a therapeutic abortion at 20 gestational weeks. On macroscopic observation of the brain of this abortus, small multiple granular protrusions over the cerebral surface were noted (Fig. 4a). There were small neuronal clusters which appeared to be migrating into the meninges through the molecular layer and the pia mater (Fig. 4b), suggestive of an initial stage of cortical dysplasia, which is a characteristic of the FCMD fetal brain [Takada et al., 1987].

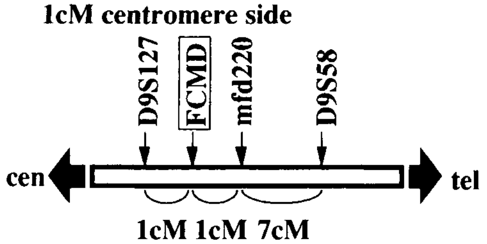
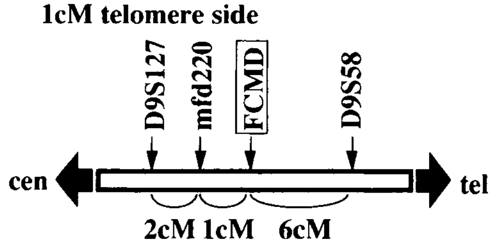
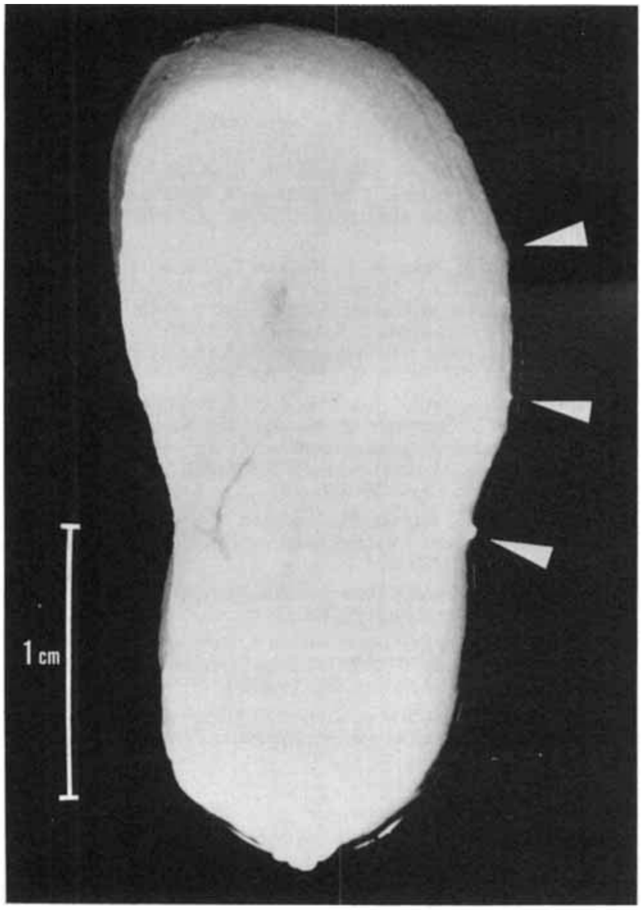
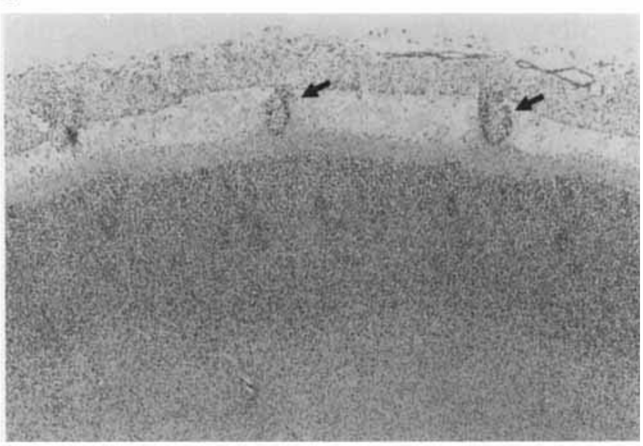
	Order of FCMD gene and mfd220	Family 1	Family 2
		possibility of **hetero zygous** carrier (FCMD **carrier**)	possibility of **homo zygous** carrier (FCMD **patient**)
I	**1cM centromere side** 	**98%**	**99%**
II	**1cM telomere side** 	**99%** (*99%)	**86%** (*75%)

Fig. 3. Phenotype probability in the fetuses. Calculation was done by hypothesizing two different location orders around the FCMD locus: 1) cen - D9S127 - (1cM) - FCMD - (1cM) - mfd220 - (7cM) - D9S58 - tel; 2) cen - D9S127 - (2cM) - mfd220 - (1cM) - FCMD - (6cM) - D9S58 - tel. Asterisk shows the probability of affectedness when the order/distance is cen - D9S127 - (1cM) - mfd220 - (1cM) - FCMD - (3cM) - CA246 - tel.



a



b

Fig. 4. Autopsied brain of the family 2 fetus. Sagittal section of a frontoparietal lesion from the right hemisphere (**a**). Small multiple granular protrusions, up to 0.5 mm in diameter, can be seen over the cerebral surface (arrowheads). Microscopically, small clusters of neuroblasts (arrows) appear to be migrating into the meninges through the surface of the molecular layer and the pia mater (parietal region, H-E stained, × 10 × 7.5) (**b**).

DISCUSSION

The haplotype analysis revealed that the fetus in family 1 was most likely a carrier (heterozygote), who had inherited the mutant FCMD allele from the mother. In family 2, the fetus had inherited an mfd220 genotype identical to that of the patient. As the mfd220 marker is nearest the FCMD locus, a likelihood of affectedness in this fetus was very high.

Considering the possibility that more recombinations may have occurred at random in the parental alleles, genotype data for D9S127, mfd220 and D9S58 loci were entered into a computer and fetal phenotype probability was calculated. This process was absolutely necessary in family 2 in order to evaluate whether the maternally derived chromosome of the fetus might not have carried the mutant allele through a recombination between the mfd220 and CA246 loci, and in this case, the fetus would have been a carrier. The calculation was done by hypothesizing two alternative occasions, when the FCMD gene is proximal to the mfd220 locus or when it is distal to the locus (Fig. 3). If a recombination occurs near the mfd220 locus, the diagnosis may become difficult. In this situation, we make a diagnosis with numerical values. In family 1, the recombination site was far from the mfd220 locus, and there was no difference between probabilities calculated with and without the risk inference. On the other hand, in family 2, a recombination occurred at a site near the mfd220 locus, and the results calculated on the basis of the two hypothesized possibilities were different (Fig. 3). In this situation, parents must be informed of the results of both risk calculations. To provide additional information, a risk in the fetus was also calculated from the tentative location data of CA246 among other markers (Fig. 3), i.e., cen - D9S127 - (1cM) - mfd220 - (1cM) - FCMD - (3cM) - CA246 - tel. Even with this calculation, the fetus in family 2 had FCMD with 75% certainty, although it was lower than the probability from D9S58-based calculation. This additional result was also provided to the parents.

After the likelihood of having another FCMD child was informed, family 1 decided to continue the pregnancy, and family 2 terminated it. The eventual outcomes confirmed that both prenatal predictions were correct. Family 1 represents a special situation in which the parents had initially been faced with tremendous familial pressure to terminate the pregnancy due to a 25% risk of having another affected child. The very low risk demonstrated by the prenatal diagnosis allayed the concerns of the grandparents, allowing the parents to opt for continuation of the pregnancy. Demands for prenatal diagnosis in FCMD families will certainly continue to increase until effective treatment measures are established. The ethical issues inherent to prenatal diagnosis should, of course, be considered carefully before a widespread establishment of prenatal diagnosis.

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